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February 8, 2007

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RE: PROJECT DIRECTOR: Middlebrooks, Bobby L.
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Enclosed please find the Final Technical Report and SF298 for the proposal entitled "Production of a DNA Vaccine Specific for the 64 kDa Protective Antigen of Erysipelothrix Rhusiopathiae". The ONR Award number is N00014-04-1-0206.

Thank you for your support. If you have questions, please feel free to contact the project director at the number above or me at 601-266-4119.

Sincerely,

Constance V. Wyldmon

Constance V. Wyldmon
Director

CVW:sj

Enclosures

cc: ONR Regional Office Atlanta
ONR 00CC, Arlington, VA
Defense Technical Information Center, Washington, DC
Naval Research Laboratory, Washington, DC

Final Report

GRANT #: N00014-04-1-0206

PRINCIPAL INVESTIGATOR: Dr. Bobby L. Middlebrooks

INSTITUTION: The University of Southern Mississippi

GRANT TITLE: Production of a DNA Vaccine Specific for the 64 kDa Protective Antigen of *Erysipelothrix rhusiopathiae*

AWARD PERIOD: December 19, 2003 through November 30, 2004

OBJECTIVES: To produce either a protein (native or recombinant) or DNA vaccine specific for *Erysipelothrix rhusiopathiae*; to produce a recombinant protein and test its antigenic similarity to the native form of the protein; to use the recombinant protein to improve existing ELISA for determining cetacean antibody titers to *E. rhusiopathiae*; and to test the DNA from the recombinant protein *in vitro* in cetacean specific cell lines for its ability to transfect the cells.

APPROACH: The gene for the protective antigen of *E. rhusiopathiae* will be inserted into a eukaryotic vector both for the production of a DNA vaccine and for large scale production of the recombinant protein (*in vitro*). The DNA vaccine will be tested in cetacean cell lines for its ability to transfect the cells. The recombinant protein will be used to improve the existing ELISA for determining the antibody titers in cetacean serum samples to *E. rhusiopathiae*.

ACCOMPLISHMENTS: The identity of the U.S. Navy Space and Naval Warfare System Center (SPAWARSYSTENS) isolate of *Erysipelothrix rhusiopathiae*, acquired from U.S. Navy Marine Mammal Program, was confirmed using previously published polymerase chain reaction (PCR) primers that amplify an *E. rhusiopathiae* specific 399 base pair 16s rRNA DNA sequence. The recombinant form of the 66kD surface protein was obtained from the cloned and expressed coding sequence of this isolate. Besides the species specific PCR, immunological and microbiological testing also confirmed the identity of the isolate as *Erysipelothrix rhusiopathiae*.

A rabbit was immunized with the 64 kDa protective antigen from *E. rhusiopathiae* purified from SDS-PAGE gels. The IgG fraction of serum from the rabbit was purified by affinity column chromatography (Protein G Sepharose® 4 Fast Flow). This antibody preparation was used to (1) purify the protective antigen (native and recombinant) by use in affinity column chromatography; (2) detect and quantitate the protective antigen in ELISA's and other immunochemical assays; and (3) select recombinant phage from the genomic library expressing the protective antigen.

The 626 amino acid primary protein sequence of the translated 1081 nucleotide sequence of the virulent Fujisawa strain of *E. rhusiopathiae* has revealed a two-domain structure. The N-terminal sequence is apparently that part of the protein that contains the immunogenic epitope(s) that have been shown to elicit a protective immune response in

swine and mice. The other domain of this protein, the C-terminal, is a glycan-binding domain that according to its sequence, is most probably involved in anchoring the protein to the cell. This is most probably the function of this region of the protein in *E. rhusiopathiae* which is the main reason why a N-terminal His-tagged recombinant form of the protein was first chosen as the expression form.

The His-tag is relatively small, poorly immunogenic, is not likely to interfere with the function of the recombinant protein and is uncharged at physiological pH. The advantage of the His-tag being neutrally charged at physiological pH is that the tag itself will not interfere with secretion. PCR cloning using a high-fidelity enzyme offered the best approach to have an adequate supply of accurate gene sequence to use in the cloning process.

Eventually a N-terminal His-tagged recombinant form of the 66kD surface protein was produced and its performance was analyzed in an ELISA. This recombinant form of the protein did not function in the ELISA as well as a non-recombinant extracted form of the protein. Attempts to produce a C-terminal His-tagged recombinant protein proved unsuccessful.

CONCLUSIONS: Affinity column chromatography proved to be a reliable method for isolating the native form of the 66kD surface protein. Given this information it is possible to produce columns of whatever size necessary to obtain the needed amount of native material from the *E. rhusiopathiae* isolate. This eliminates problems associated with comparing between native and recombinant forms of the protein. However, it does not eliminate the problem of having a steady supply of the protein for the many uses (ELISA for antibody titer determination, vaccines, western blot or ELISPOT) that marine mammal veterinarians wish to take advantage of in treating their animals (captive or wild). Given that neither the N-terminal nor C-terminal His-tagged recombinant protein proved useful, it is necessary to consider whether that was due to (1) the limited amount of time for this project; (2) the limited experience within the laboratory for this type of work; or (3) the ability of this protein in a recombinant form to function correctly with this type of tag. Regardless of the reason for not obtaining the recombinant protein, without it there was not any way to accomplish the objective of producing a vaccine or of testing the materials on the cetacean specific cell lines already produced and present in this laboratory.

However, it is important to note that the gene isolated for the 66kD surface protein was shared with other researchers working on the production of DNA vaccines for cetaceans. It is also important to note that the ELISA work done through this project has determined antibody titers (specific for *E. rhusiopathiae*) that has assisted projects associated with the John G. Shedd Aquarium (in conjunction with NOAA/NMFS) with samples from both captive and wild (catch/release) dolphins; in vaccine trials at SeaWorld Adventure Parks; and various smaller facilities (nationally and internationally) with samples from their animals.

SIGNIFICANCE: The monetary value of captive cetaceans, whether in the U.S. Navy program or at an oceanaria or aquaria, establishes the need for development and evaluation of efficacious vaccines (protein or DNA). *E. rhusiopathiae* is a highly pathogenic bacteria that is known to cause deaths in wild and captive cetaceans with little

or no indications of illness prior to death; thus, limiting the veterinarians from intervening with antibiotics that could eliminate the infection. Development of a vaccine specific for the protective protein of *E. rhusiopathiae* would decrease the chances of one of the Navy's (or others') animals succumbing to this organism.

PATENT INFORMATION: No patents applied for or received.

AWARD INFORMATION: Non-applicable

PUBLICATIONS AND ABSTRACTS (for total period of grant):

1. Osgood, Robert C. (2004) An investigation of *Erysipelothrix rhusiopathiae* isolates affecting marine mammals and the cloning, expression and evaluation of a putative recombinant 66kD immunoprotective surface protein. Dissertation defense presented November 2004. The University of Southern Mississippi, Hattiesburg, MS.

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